Hydrochloric Fractions in Hedley Fractionation May Contain Inorganic and Organic Phosphates

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ABSTRACT

Accurately characterizing phosphorus (P) forms is a prerequisite to developing effective remediation strategies to minimize the adverse environmental impact of agricultural expansion. Modified or unmodified Hedley sequential fractionation procedures have been widely used for characterizing P forms in soil, animal manure, and biosolids. Hydrochloric acid (HCl) fractions in these procedures have often been assumed to contain no organic P. As a result, many researchers for the last two decades have measured concentrations of inorganic P in HCl fractions without measuring organic P. In this study, we measured colorimetrically concentrations of inorganic P in untreated (control) HCl fractions after 3-phytase incubation or oxidative autoclaving of these fractions. Phosphorus concentrations in some samples were also determined by inductively coupled plasma optical emission spectroscopy. The increases in measured P concentrations, compared with the controls, were attributed to organic P in these fractions. Data for 15 soil and eight manure samples indicated that, although HCl fractions of some samples contained negligible amounts of organic P, others contained significant amounts of organic P. The concentrations of organic P were greater than inorganic P in one soil sample and two manure samples. We recommend that organic P in HCl fractions be measured experimentally to determine whether the HCl fraction contains inorganic and organic P. Identification of possible organic P species contained in HCl fractions of the Hedley sequential fractionation schemes would provide more accurate and comprehensive knowledge of the fates and interchanges of P forms in soil and animal manure under various environmental conditions.

PHOSPHORUS is an essential nutrient for plant growth and development. It is added to cropland in the form of fertilizers or animal manures to maintain its availability for profitable crop production. However, P runoff from agricultural land has been identified as a nonpoint source of pollution and the cause of accelerated eutrophication in surface waters (Sharpley et al., 1999). It is expected that the first 50 years of the 21st century may be the final period of rapid global agricultural expansion accompanied by a 2.4- to 2.7-fold increase in nitrogen- and P-driven eutrophication of terrestrial, freshwater, and near-shore marine ecosystems (Tilman et al., 2001). Thus, minimizing eutrophication will be a great challenge. Because P exists in nature in various forms with

different degrees of solubility and bioavailability, accurately characterizing these P forms and their transformations in soil/water environments is a prerequisite to developing effective remediation strategies to minimize adverse environmental impacts.

Sequential fractionation with dilute bases, acids, and organic solvents has been long used to characterize P in soil and animal manure (Barnett, 1994; Bowman and Cole, 1978; Chang and Jackson, 1957; Hedley et al., 1982b; Peperzak et al., 1959). The Hedley et al. (1982b) fractionation scheme has been widely used for investigating the forms and transformations of soil P under various environmental conditions (Cross and Schlesinger, 1995; Schoenau et al., 1989; Tiessen et al., 1984; Trasar-Cepeda et al., 1990). This procedure separates soil P into anion exchange resin, $0.5 \, M$ $NaHCO_3$, $0.1 \, M$ $NaOH$, $1 \, M$ HCl, and residual fractions. An additional step of $0.1 \, M$ $NaOH$ with sonication following $0.1 \, M$ $NaOH$ extraction is occasionally included to explore the P held at the internal surfaces of soil aggregates (Beck and Sanchez, 1994; Hedley et al., 1982a; Schmidt et al., 1996; Zheng et al., 2002). Alternatively, the anion exchange resin fraction has been replaced by a H_2O extraction to investigate P distributions in biosolids (Ajiboye et al., 2004; Sui et al., 1999), animal manure and composts (Ajiboye et al., 2004; Dou et al., 2000; Dou et al., 2003; He and Honeycutt, 2001; He et al., 2004; Sharpley and Moyer, 2000; Wienhold and Miller, 2004), and relevant amendments or soils. Hedley et al. (1982b) measured inorganic and organic P (difference between total P and inorganic P) present in water, $0.5 \, M$ $NaHCO_3$, and $0.1 \, M$ $NaOH$ fractions. They, however, assumed that only inorganic P exists in the $1 \, M$ HCl fraction, which has led many researchers to measure only inorganic P in HCl fractions without considering any organic P (e.g., Cross and Schlesinger [1995]; Schoenau et al. [1989]; Sharpley and Moyer [2000]; Sui et al. [1999]; Tiessen et al. [1984]; Trasar-Cepeda et al. [1990]). There are only a few reports (Dou et al., 2000; Dou et al., 2003; He and Honeycutt, 2001; He et al., 2003; Wienhold and Miller, 2004) in which the concentration of total P in all four fractions were measured. He et al. (2003) reported a difference in inorganic P and total P of 29% in the HCl fraction of swine manure. Dou et al. (2003) reported significant amounts of organic P in the HCl fractions of swine and broiler samples, but they presented no specific quantitative data. Furthermore, none of the five publications have raised the issue that organic P should not be presumably excluded from HCl fractions; rather, it should be

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Abbreviations: ICP–AES, inductively coupled plasma atomic emission spectroscopy; P_i, soluble inorganic orthophosphate determined by a molybdenum blue method.

determined experimentally. In the meantime, other researchers continue to assume that 1 M HCl fractions in the Hedley sequential fractionation scheme contain only inorganic P (Ajiboye et al., 2004; McDowell and Stewart, 2005).

With the importance of organic P in the environment being recognized (Turner et al., 2005), the ambiguity of whether organic P should be categorically excluded from the sequentially extracted HCl fractions has to be clarified experimentally. In this study, we determined the concentrations of inorganic P, enzymatically hydrolyzable organic P, and total P of sequentially extracted 1 M HCl fractions in 15 soil samples and eight animal manure samples to evaluate the validity of categorically excluding organic P components in 1 M HCl fractions. Concentrations of P and major metal elements (Ca, Mg, Al, and Fe) in some fractions were also determined by inductively coupled plasma atomic emission spectroscopy (ICP–AES) to explore the relationship between the extracted P and these elements.

MATERIALS AND METHODS

Soil and Manure

Soil samples were obtained from Alabama, Maine, and Oregon (Table 1). These samples were sieved (2 mm), air-dried, and stored at room temperature until use. We chose these soil samples with various field and laboratory treatments because the impacts of such treatments on soil P status have been frequently evaluated using the sequential fractionation.

Soil samples from Maine and Oregon were used in a 150-d laboratory incubation. Treatments included manured and unmanured soils run in quadruplicate. Manure provided the equivalent of 22 mg P kg^{-1} dry soil. The dry weight of field moist soil added to incubation vessels was 50 g. Incubation vessels were kept at 25°C and at a moisture level of 0.27 kg kg^{-1} (determined gravimetrically). Subsamples (1 g) were taken at the beginning (0 d) and the end of the incubation (150 d). Subsamples were stored at −20°C until use and then air dried.

Two swine (*Sus scrofa*) manures, two cattle (*Bos taurus*) manures, and two poultry litters were collected from commercial farms in Maine from June 1998 to September 2001. Manure samples were homogenized, freeze-dried, ground to pass a 2-mm sieve, and stored at −20°C until use. One poultry litter had been kept at 4°C for 3 yr before the sample treatment. The additional two poultry litter samples were collected from commercial farms in Alabama during December 2003.

Table 1. Soil samples used in the study.

Sample	Location	Soil type	Cropping system	Field treatment	Fertilization
PLS0	**Alabama**	**Hartselle†**	**Pasture**	**None**	**None**
PLS15	**Alabama**	**Hartselle**	**Pasture**	**None**	**15-yr PL#**
PLS20	**Alabama**	**Hartselle**	**Pasture**	**None**	**20-yr PL††**
NTSoy	**Alabama**	**Decatur‡**	**Soybean**	**No-tilled**	**Synthetic**
NTCorn	**Alabama**	**Decatur**	**Corn**	**No-tilled**	**Synthetic**
CTSoy	**Alabama**	**Decatur**	**Soybean**	**Mold board**	**Synthetic**
CTCorn	**Alabama**	**Decatur**	**Corn**	**Mold board**	**Synthetic**
Maine	**Maine**	**Unnamed§**	**Potato**	**Mold board**	**Synthetic**
Oregon	**Oregon**	**Adkins¶**	**Grass**	**None**	**None**

† Fine sandy loam, siliceous, thermic, Typic Hapludults.
‡ Fine, kaolinitic, thermic, Rhodic Paleudult.
§ Coarse-loamy, mixed, frigid Typic Haplorthod.
¶ Coarse-loamy, mixed, mesic, Xerollic Camborthid.
\# Poultry litter was applied for 15 yr at an annual rate of 3.63 Mg ha^{-1}.
†† Poultry litter was applied for 20 yr with annual rate of 1.36 Mg ha^{-1}.

These poultry litters were air dried, homogenized, ground to pass through a 0.5-mm sieve, and stored at room temperature until use.

Preparation of Sequentially Extracted 1 M HCl Fractions

Each Alabama soil sample (1.5 g dry weight) was extracted with 30 mL of deionized water. Extraction was performed at 22°C by placing the sample tubes horizontally on the platform of an orbital shaker (250 r min^{-1}). After a 2-h extraction, the tubes were centrifuged at 14 000 × g for 30 min at 4°C, and the supernatant was removed. To reduce contamination of residual extractant and extracted P in the pelleted portion to be used in the next extraction, the residues (pellets) were washed with 5 mL of water, and the washing supernatant was discarded after centrifuging. Using the same procedure, the residues were sequentially extracted with 30 mL of 0.5 M NaHCO₃ (pH 8.5), 0.1 M NaOH, and 1 M HCl for 16 h each. Only the 1 M HCl extracts were saved for this study. Sequentially extracted 1 M HCl fractions of undried Maine and Oregon soils (equivalent to 1 g dry soil in 25 mL extractant) and animal manure (0.25 mg in 25 mL extractant) were prepared in the same way.

All 1 M HCl fractions were filtered through a 0.45-μm membrane. The pH of the filtered 1 M HCl fraction was adjusted to 2.5 by mixing one part of the fraction with two parts of 1 M glycine solution. Oregon soil samples were further diluted with an equal volume of deionized water. Poultry litter samples were further diluted by adding 7.75 mL of deionized water to the mixture of 0.75 mL of extract and 1.5 mL of glycine solution (10 mL total volume). No further dilution was done on other pH adjusted extracts. The pH-adjusted and properly diluted extracts were immediately used for inorganic P and hydrolyzable organic P determinations as described below. The remaining extracts were also kept at −20°C for later element analysis.

Phosphorus Determination

Each 0.45 mL of pH-adjusted and properly diluted extract was mixed with 0.5 mL of deionized water or 0.5 mL of *Aspergillus ficcum* 3-phytase solution (final concentration 0.25 U activity mL^{-1} mixture). These mixtures were incubated on a rotating shaker (250 r min^{-1}) at 37°C for 1 h. Soluble inorganic orthophosphate (P_i) in these incubation mixtures (0.2 mL each) was directly quantified by a molybdenum blue method (He and Honeycutt, 2005) modified from an earlier method (Dick and Tabatabai, 1977) by changing the measured wavelength to 850 nm and adding of 2% sodium dodecyl sulfate. This method was developed for accurate determination of P_i, whereas other molybdenum blue methods determine a loosely defined "molybdenum-reactive P," which may include some labile organic P and condensed P_i (such as pyro- and polyphosphate) (Dick and Tabatabai, 1977). The final pH in molybdenum blue methods is critical for accurate P determination. The useful ranges are pH 0.4–0.9 and pH 2.8–4.6 with ascorbic acid as reducing agent (Lowry and Lopez, 1946). The pH in our P determination was stable and around pH 0.5 due to high concentrations of acids in Reagent A (0.1 M ascorbic acid and 0.5 M trichloric acid). Controls with a pH-adjusted/diluted blank extractant and/or enzymes were included. The P_i concentration in samples with deionized water was designated as inorganic P. Enzymatically hydrolyzable organic P was the increase of P_i concentration after 3-phytase incubation. Total P was determined in the same way after persulfate digestion of diluted extracts (Rowland and Haygarth, 1997;

Schoenau and Huang, 1991). Specifically, each 0.2 mL of pH-adjusted and properly diluted extract was mixed with 0.4 mL of 0.5 M H_2SO_4 and 16 mg of $K_2S_2O_8$ and autoclaved for 1 h. After it cooled, 0.2 mL of the autoclaved sample was taken out for P determination. To digest all organic P in poultry litter fractions, the amount of $K_2S_2O_8$ was doubled in enzymatically hydrolyzed aliquots. Total P, Ca, Mg, Fe, and Al in selected diluted extracts were determined with ICP–AES (Plasma 400 Emission Spectrophotometer; PerkinElmer, Norwalk, CT).

Statistical Analysis

All data are presented as the average of three to six replicates, and error bars represent standard deviations. ANOVA-single factor analysis was used to evaluate the differences of organic P determined by different methods or treatments.

RESULTS AND DISCUSSION

Phosphorus Forms in 1 M HCl Fractions of Alabama Field Soils

Detectable levels of inorganic P and total P were not observed in the HCl fraction of the pastoral Harstelle soil without poultry litter application. However, 1.7 mg P kg^{-1} dry soil was detected after enzymatic incubation (Fig. 1). Yearly application rates in the 15-yr manure study (54.45 Mg poultry litter ha^{-1}) were nearly double those of the 20-yr manure study (27.2 Mg poultry litter ha^{-1}). As a result, higher concentrations of P were measured in the HCl fraction of the soil with a 15-yr poultry litter application history compared with the soil with a 20-yr application history (Fig. 1). Enzymatic incubation increased P_i concentration by 51% in the 15-yr soil sample and by 16% in the 20-yr soil sample. Autoclaving increased P_i detected by 6% in the 15-yr soil sample and by 20% in the 20-yr soil sample. These observations demonstrate that, in addition to inorganic P, there were also organic P forms in the HCl fractions of these soils.
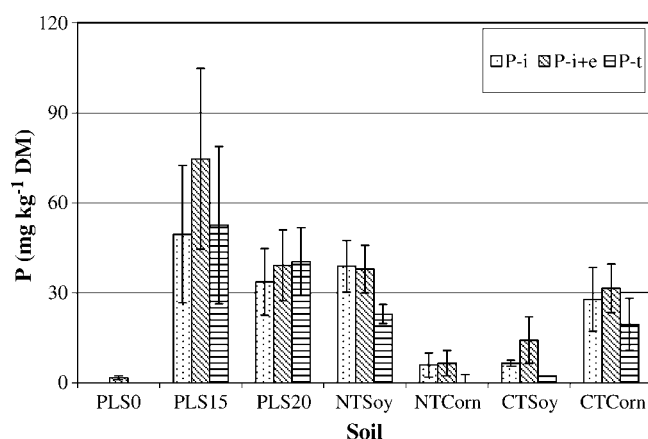
Fig. 1. Concentrations of inorganic (P-i), inorganic and enzymatically hydrolyzable organic P (P-i+e), and total P (P-t) in sequentially extracted 1 M HCl fractions of Alabama pastoral Hartselle soils with 0-yr (PLS0), 15-yr (PLS15), and 20-yr (PLS20) poultry litter applications and in Alabama arable Decatur soils with no-tilled soybean (NTSoy), no-tilled corn (NTCorn), conventional soybean (CTSoy), and conventional corn (CTCorn) cropping systems. Values are averages $\pm$ standard deviations ($n = 6$ for poultry litter soils; $n = 4$ for others).

The standard deviation seems to be greater than the increase due to enzymatic incubation or autoclaving. In fact, there are multiple sources of variation (such as less uniform soils in the field and experimental interference/contamination caused by known and unknown factors in the laboratory processes). For example, the soil samples with a 15-yr poultry application history were randomly collected from three locations. Because no tillage was done on these pastures, poultry litter was unevenly distributed on the soil, resulting in an average P_i of 49.5 mg P kg^{-1} dry soil and a large standard deviation (25.5). Enzymatic incubation increased the P_i concentration to 74.6 $\pm$ 32.6 mg P kg^{-1} dry soil (Fig. 1). The increase in P_i due to enzymatic incubation does not seem to be significant due to the large standard deviation. On the other hand, the individual concentrations of P_i in each 1 M HCl fraction of the three locations were 49.6 (duplicate extraction data for the same sample), 74.9, and 23.9 mg P kg^{-1} dry soil, respectively. The concentrations after enzymatic incubation were 65.4, 110.8, and 47.7 mg P kg^{-1} dry soil, respectively. Individual data at each location revealed increases in P_i measured after enzymatic incubation. Therefore, the existence of organic P in these soil samples should not be undermined by the nonhomogeniety in the soil samples.

The 1 M HCl extracts from the Alabama arable Decatur soils under no-tilled cropping managements contained inorganic P only (Fig. 1). However, enzymatically hydrolyzable organic P species were observed in soils with conventional tilled (moldboard plow) cropping managements. There was a higher P concentration in the HCl fraction of soils with the no-tilled soybean cropping system than that with the conventionally tilled soybean system, although the trend was reversed between no-tilled and conventional corn cropping systems. We do not know what caused the differences between the soybean and corn systems. In all four soil samples, the concentration of total P measured after autoclaving was lower than those of inorganic P (Fig. 1). We believe that the major cause for this contradiction was the different degrees of background interference yielded from medium matrix changes in extracts after autoclaving on P_i determination.

Phosphorus Forms in 1 M HCl Fractions of Soils Amended with Dairy Manure

There was 140 mg inorganic P kg^{-1} dry soil in the HCl fraction of the unamended Maine sandy loam (Fig. 2). No apparent increase in P_i was observed after enzymatic incubation or autoclaving. Amendment with dairy manure did not change the predominance of inorganic P in the HCl fractions of this soil (Fig. 2) due to the low application rate. Incubation of the soil at 25°C for 150 d did not change the concentrations or patterns of the predominant forms of P. In contrast, there was a higher concentration of inorganic P in the HCl fractions of Oregon Adkins loamy sand with 752 mg inorganic P kg^{-1} dry soil (Fig. 2). Enzymatic incubation released 21 mg organic P kg^{-1} dry soil, and autoclaving released 244 mg organic P kg^{-1} dry soil (Fig. 2). These data suggest that about 25% of P in the HCl fractions of the
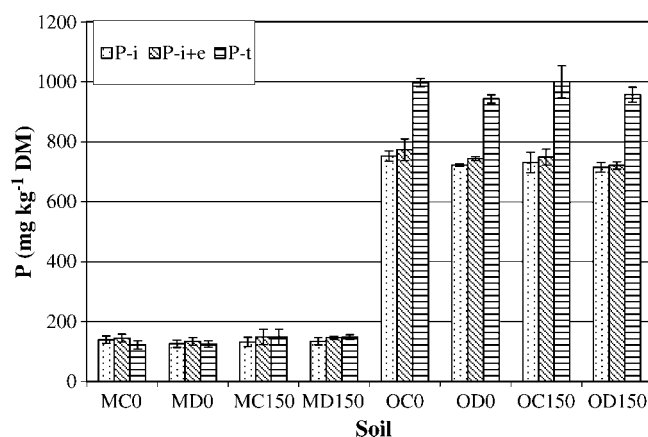
Fig. 2. Concentrations of inorganic (P-i), inorganic and enzymatically hydrolyzable organic P (P-i+e), and total P (P-t) in sequentially extracted 1 *M* HCl fractions of soils mixed with or without dairy manure. Maine unnamed soils without dairy slurry Day 0 (MC0) and with dairy slurry Day 0 (MD0) after a 150-d incubation (MC150 and MD150). Oregon Adkins soils without dairy slurry Day 0 (OC0) and with dairy slurry Day 0 (OD0) and after incubated 150 d (OC150 and OD150). Values are averages ± standard deviations (*n* = 4).

Oregon soil was organic P, mainly in enzymatically non-hydrolyzable forms. Amendment with dairy manure somehow reduced inorganic P content by 30 mg P kg^{-1} dry soil, but organic P content was unchanged (Fig. 2). As observed for the Maine soil, short-term incubation did not affect the HCl-extracted P levels in the Oregon soil.

Phosphorus Forms in 1 *M* HCl Fractions of Animal Manure

The concentrations of HCl extractable P in the two swine manures were quite different (Fig. 3). Extracts of swine manure 1 contained 3660 mg inorganic P kg^{-1} dry matter, with 2500 g organic P kg^{-1} dry matter released by enzymatic hydrolysis, or 2050 mg organic P kg^{-1} dry matter by autoclaving. The HCl extracted inorganic P in
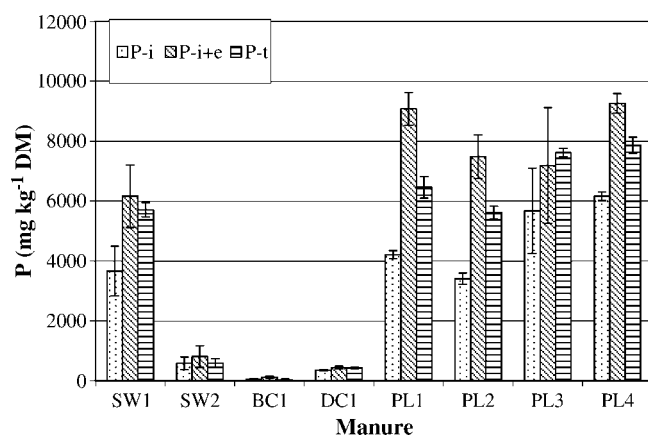

Fig. 3. Concentrations of inorganic (P-i), inorganic and enzymatically hydrolyzable organic P (P-i+e), and total P (P-t) in sequentially extracted 1 *M* HCl fractions of swine manure (SW1 and SW2), beef cattle manure (BC1), dairy cattle manure (DC1), and poultry litter (PL1, PL2, PL3, and PL4). Values are averages ± standard deviations (*n* = 3).

swine manure 2 was 580 mg P kg^{-1} dry matter. Enzymatic hydrolysis released 230 mg organic P kg^{-1} dry matter, although P_i determined after autoclaving increased by only 10 mg inorganic P kg^{-1} dry matter. Both cattle manures contained low concentrations of HCl-extracted inorganic P. Enzymatic hydrolysis increased P_i by 35 to 123%, but the absolute amounts were relatively low (68–88 mg P kg^{-1} dry matter) (Fig. 3). All four poultry litters contained high concentrations of inorganic P and organic P in 1 *M* HCl fractions (Fig. 3). The organic P contents were even higher than those of inorganic P in poultry litter 1 and 2. The average of P_i of the four samples was 4865 mg P kg^{-1} dry matter without treatment, 8253 mg P kg^{-1} dry matter after enzymatic incubation, and 6891 mg P kg^{-1} dry matter after autoclaving.

Unlike soils, there have been several reports on the determination of inorganic P and total P in HCl fractions of animal manures using a modified Hedley sequential fractionations (Dou et al., 2003; He and Honeycutt, 2001; He et al., 2003; Wienhold and Miller, 2004). Among them, He et al. (2003) reported a difference between inorganic P and total P of 29% in swine manure. Dou et al. (2003) reported that HCl fractions of their swine and broiler samples contained more organic P than inorganic P, which is contrary to their dairy manure samples. However, no specific values were presented. He and Honeycutt (2001) and Wienhold and Miller (2004) found relatively low amounts of HCl-extractable inorganic P and total P in cattle manure and swine manure. Several other reports (Dou et al., 2000; He et al., 2004; Leinweber et al., 1997) have presented data of HCl- or H$_2$SO$_4$–extractable total P in animal manure. These data show that (dairy) cattle manure contained relatively lower amounts of HCl-extractable P (<500 mg P kg^{-1} dry matter), whereas poultry litter contained much higher amounts of HCl- or H$_2$SO$_4$–extractable P (>4000 mg P kg^{-1} dry matter). Swine manure could contain a high (>4000 mg P kg^{-1} dry matter) (Leinweber et al., 1997) or low (<500 mg P kg^{-1} dry matter) (He et al., 2004) concentration of acid-extractable P. The variability of P concentrations in these manure sources confirms the need to measure organic P in HCl fractions. The results of this work are consistent with these previously published studies. The general level of HCl-extractable P in the modified Hedley sequential fractionation was in the order of cattle manure < swine manure < poultry litter.

Sharpley and Moyer (2000) investigated P forms in manure and compost by the modified Hedley sequential fractionation. Their data on HCl-extractable inorganic P of dairy manure (60 mg P kg^{-1} dry matter), poultry litter (4776 mg P kg^{-1} dry matter), and swine slurry (3294 mg P kg^{-1} dry matter) are similar to the present data set (Fig. 3). Organic P in their samples was not measured due to the untested assumption in the original work of Hedley et al. (1982b). The findings in this work and He et al. (2006) suggest that the poultry manure/litter samples and probably swine manure samples used in their study might have contained significant amounts of HCl-extractable organic P. Similarly, Ajiboye et al.

(2004) characterized P forms in fresh and oven-dried biosolids, cattle manure, and swine manure. Neglecting the possibility of organic P in the HCl fraction, data may be of limited use in defining the P transformations in those manures affected by different pretreatment processes. Biosolids and composts were not included in the present study. It is reported, however, that biosolids (Ajiboye et al., 2004; Sui et al., 1999) and manure composts (Sharpley and Moyer, 2000) contain high amounts of HCl-extractable inorganic P (>25% of total P). It would be of interest to determine if significant amounts of organic P exist in these HCl fractions.

Comparison of Organic Phosphorus Determined by Different Approaches

A comparison of organic P determined by the increase of P_i after three treatments of 1 M HCl fractions of selected samples is listed in Table 2. Organic P was detected positively in all but one sample by enzymatic incubation. Negative values appeared in four samples with the oxidative autoclaving treatment and four samples with ICP–AES measurements. Apparently, the inconsistent or altered background interferences between the colorimetric method for P_i and ICP–AES or the oxidative autoclaving treatment for total P led to negative values. The observation of some negative values might partly explain why the organic P concentration in the HCl fractions was omitted in so many previous publications. Addition of enzyme(s) kept the medium matrixes of extracts unchanged. The similar background

interference in the presence and absence of the enzymes made the increase of P_i with enzymatic incubation credible. Increases in P_i should be attributed to the presence of organic P in these extracts. Thus, enzymatic hydrolysis provided another means to explore the existence of organic P in these samples. The disadvantage was that only enzymatically hydrolyzable P was determined by this method. The organic P in a sample might have been underestimated if the amount of nonhydrolyzable P was significant, as in the case of the Oregon soil (Fig. 2 and Table 2).

Major Metal Elements Extracted by 1 M HCl

Hedley et al. (1982b) proposed that 1 M HCl dissolves calcium phosphate and some P occluded within sesquioxides. In the soil extracts, the contents of all four major elements—Ca, Mg, Fe, and Al—were higher than total P contents (Fig. 4). Except for the soil with conventional corn cropping management, the increase in Ca was parallel to the increase of total P. However, significant increases of Al and Fe appeared with increases of total P in some soils. Thus, calcium-bound P and sesquioxides (Al and Fe)-relevant P may be present in 1 M HCl extracts of soils.

In the animal manure samples, the Ca content was much higher than those of Fe, Al, and Mg (Fig. 5). The cumulative concentrations of Fe, Al, and Mg were lower than total P concentration in three of the four manure samples, indicating the dominance of Ca-bound P in the HCl fractions of animal manure. Solid-state speciation of phosphate in poultry litter conducted by Peak et al. (2002) has found no thermodynamically stable calcium phosphate phases (such as hydroxylapatite) in poultry litter. In the Hedley fractionation procedure, most of the Ca-bound P was in the 1 M HCl fractions. Thus, the portion of P in animal manure extracted with 1 M HCl could be more labile than the same portion of P in soils. Therefore, these manure P species could be transformed to a more stable hydroxylapatite phase or

Table 2. Comparison of organic P determined by enzymatic hydrolysis (P-o1), autoclaving (P-o2), and inductively coupled plasma (P-o3).†

	P-o1	P-o2	P-o3
	mg kg^{-1} dry matter		
PLS0	1.1a‡	0.4a	ND§
PLS15	25a	3.0b	ND
PLS20	5.7a	7.6b	−0.9a
NTSoy	−0.9a	−16b	ND
NTCorn	0.5a	−5.1b	ND
CTSoy	7.7a	−4.2b	ND
CTCorn	3.7a	−8.4b	−5.7b
MD150	12a	14a	−0.5a
OD150	6.5a	243b	88c
SW1	2502a	2046a	2304a
SW2	228a	8a	ND
BC1	69a	62a	ND
DC1	88a	74a	−78b
PL1	4868a	7531b	6281a
PL2	4075a	6166b	ND
PL3	1489a	3345a	1562a
PL4	3102a	5243b	ND

† Hartselle soils with 0-yr (PLS0), 15-yr (PLS15), and 20-yr (PLS20) poultry litter applications and in Alabama arable Decatur soils with no-tilled soybean (NTSoy), no-tilled corn (NTCorn), conventional soybean (CTSoy), and conventional corn (CTCorn) cropping systems. Maine un-named soils without dairy slurry Day 0 (MC0) and with dairy slurry Day 0 (MD0) after a 150-d incubation (MC150 and MD150). Oregon Adkins soils without dairy slurry Day 0 (OC0) and with dairy slurry Day 0 (OD0) and after incubated 150 d (OC150 and OD150). The four soil samples were mold-board plow in corn (CTCorn), Alabama pastoral Hartselle soils with 20-yr poultry litter applications (PLS20), Maine soil amended with dairy slurry at 150-d (MD150), and Oregon soil amended with dairy slurry at 150 d (OD150).

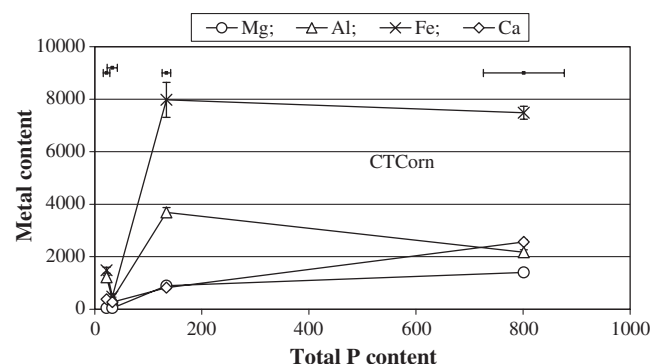‡ Different letters within the same row indicate significant differences at α = 0.05.

§ ND, not determined.

Fig. 4. Relationship of metal contents and total P contents in HCl fractions of soils. The four soil samples were mold-board plow in corn (CTCorn), Alabama pastoral Hartselle soils with 20-yr poultry litter applications (PLS20), Maine soil amended with dairy slurry at 150-d (MD150), and Oregon soil amended with dairy slurry at 150 d (OD150) (see Fig. 1 and 2) with the increasing order of P contents (m mol kg^{-1} dry soil). Horizontal bars represent standard deviations of P contents. Vertical bars represent standard deviations of metal contents.
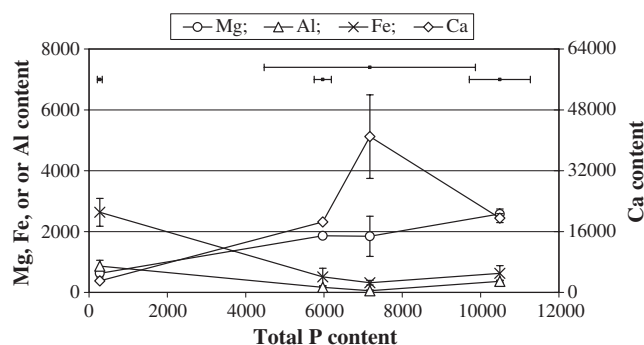
Fig. 5. Relationship of metal contents and total P contents in HCl fractions of animal manure. The four manure samples were dairy cattle manure (DC1), swine manure (SW1), and poultry litter (PL1 and PL3) (Fig. 3) with the increasing order of P contents (m mol kg^{-1} dry soil). Horizontal bars represent standard deviations of P contents. Vertical bars represent standard deviations of metal contents.

become bioavailable in soil. It would be of interest to explore how environmental factors affect the P transformations and dynamics once animal manure is applied to soils.

CONCLUSIONS

Different versions of the Hedley sequential fractionation scheme have been widely used for characterizing P forms in soil, animal manure or compost, and biosolids. The fractions extracted by 1 M HCl in these procedures have traditionally been assumed to contain inorganic P, neglecting the presence of organic P. In this work, we determined inorganic P concentrations in 1 M HCl fractions of 15 soil samples and eight animal manure samples. We also measured the increases in inorganic P concentrations in these samples after enzymatic incubation and oxidative autoclaving. Our data indicate that although some samples contained negligible amounts of organic P in HCl fractions, other samples contained significant amounts of organic P. The concentrations of organic P were even greater than those of inorganic P in a soil and two manure samples. Thus, we believe that results and findings would be more definitive if the significance/insignificance of organic P in the HCl fractions were experimentally determined. Elemental analysis and enzymatic hydrolysis results suggest that some of the organic P species in the sequentially extracted HCl fractions were Ca-bound hydrolyzable organic P. Alleviating this methodologic misconception would allow researchers to more accurately investigate the P compositions within the Hedley sequential fractionation method, thus gaining novel insights into the fate and transformation of P species in soil and animal manure under various environmental conditions.

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